**Gene Polymorphisms that Predispose to Infectious Diarrhea**
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The triangle of pathogen, host, and the environment encompasses age, immunity, race/ethic factors in the host; travel, migration, ecological niches, and industrialization in the environment; and mutations, acquisitions, and losses of genetic elements in the pathogen. Human genetics encompasses functional variation in the human genome, which has evolved to facilitate defense against infectious pathogens. In most cases, the susceptibility to infection is polygenic, rather than being limited to just one or a few genes. It appears that genes expressed in response to infection evolve at a higher rate, and that the genomes of both pathogen and host evolve in response to a changing environment. The magnitude of the effect is illustrated by familial studies where close physical proximity in a common environment, for example in sibling studies, has shown that the genetic contribution increases the risk by 1.5 to 5 times. There are also inter-racial and inter-population differences. Susceptibility to infection is inherited, and the early death of a parent from infectious disease carries a six-fold risk of a similar fate for the offspring. The phenomenon is more clearly demonstrated in chronic parasitic, fungal, and mycobacterial infections. Furthermore, genetics determines the severity of infection, in addition to susceptibility. The reasons for studying host genetics include understanding pathogenesis, resistance mechanisms, risk prediction and behavior modification, risk assessment, improved use of vaccines and therapeutics, and the use of 'genetic profiling' to inform individualized prevention and treatment of diseases, rather than identification of new therapeutic targets and a deeper understanding of pharmogenomics.

Approaches to studying human host genetics and disease susceptibility include association studies in case control studies and mapping genes in susceptible mice, combined with a search for homologs in humans and genetic linkage studies in families, where genes can be identified without knowing their function a priori.

A useful model to study the contribution of genetics to susceptibility to gastrointestinal infection is Travelers' diarrhea which affects 40% -60% of individuals at risk, including considerable morbidity among troops and tourists both in acute disease and in terms of chronic complications, such as post-infectious irritable bowel syndrome, reactive arthritis and others.

Bacterial pathogens account for 85% of travelers' diarrhea cases, of which the etiological agents are most commonly enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), *Shigella, Campylobacter jejuni, Salmonella, Aeromonas, Plesiomonas,* and *Vibrio cholerae*. *Cryptosporidium* and *Giardia* are the most common parasites.

ETEC is a non-invasive cause of secretory diarrhea ETEC elaborates two major toxins, namely the labile toxin (LT, a homolog of CT, expressed by cholera toxin), which bind gangliosides GM1 and GD1b, and the heat stable toxins (ST), which bind and activate guanylate cyclase.

EAEC causes secretory and inflammatory diarrhea primarily in children, patients with AIDS, travelers, and ii associated with foodborne outbreaks of disease. When incubated with cultured human intestinal epithelial cells of the HEp-2 line, there is a characteristic 'stacked brick' pattern that is associated with the presence of a 60 MD plasmid. EAEC possesses the several putative virulence factors, namely the EAEC enterotoxins, which are distinct from LT or CT and include the plasmid-encoded toxin (Pet), heat stable enterotoxin (EAST-1) as well as other virulence factors such as, *Shet-1, Shet-2;* dispersin (*asp2)*, flagellin (*fliC*), mucus biofilm, and the adherence fimbriae AAF/I and AAF/II. The receptor for a subset of EAEC adherence is Decay Accelerating Factor (DAF, CD55), which is the receptor for Dr adhesins such as AAF/II. CD55 is known to down-regulate complement activity. In addition, CD55 contains 10 epitopes of the Cromer Blood groups which possess 40 single nucleotide polymorphisms. Furhtermore, Dr+ organisms bind to CD55 at SCR 2,3,4. The infection of the intestinal epithelia by EAEC results in increased levels of IL-8 via NF-κβ activation, which results from elaborateinteractions between flagellin and TLR5.

Gastrointestinal infection can be conceptualized into three stages, first during the earliest stage of susceptibility, pili, adhesins, and colonization factors interact with host factors such as receptors, innate immunity components, opsonins, defensins, collectins, TLRs, and organisms are subject to pre-existing immunity. The injury phase is characterized by expression of virulence factors, and host responses encompassing inflammatory cytokines, chemokines, prostaglandins, kinins, and functional activation of targets. The recovery/healing phase is characterized by anti-inflammatory cytokines, growth factors, and specific humoral, cellular, and secretory immunity. At each level the response can be impacted by host genetic variation.

The study objectives were to conduct a SNP candidate association study to determine the susceptibility and severity of travelers' diarrhea, the fecal cytokine response to infection, the relative contribution of host pathogen factors have on disease, and evaluate post-travel chronic diarrhea and IBS-like symptoms. The study involved US travelers to Guadalajara, Jalisco and Cuernavaca, Morelos, Mexico in 2002-05. Volunteers were enrolled within 72 hrs. of arrival, and blood was collected both on arrival and departure for serology and DNA studies. During the stay, subjects recorded their symptoms in a diary and they were instructed to contact the clinic at any time of day or night in case of diarrhea. The laboratory studies included evaluation of diarrheal stools for mucus, fecal leukocytes, and occult blood, and the presence of *Shigella, Salmonella, Campylobacter,* ETEC (LT and ST), EAEC, *C. difficile, Entamoeba, Giardia, Cryptosporidium, Aeromonas, Plesiomonas,* and *Providencia.*

Genetic studies started with extraction of DNA from blood, and analysis for single nucleotide polymorphisms by PCR-pyrosequencing or direct sequencing. Fecal cytokines were measured by ELISA, and clinical outcomes and associations determined by the $\chi 2$ test. To date >1,000 subjects have been enrolled, and 698 completed the study with 6 month follow-up. Females constituted 55% , white 74%, Hispanic 20%, Asian 2%, African American 2%, and Native American <1 %. The mean length of stay was 31d (7-45), and travelers' diarrhea was seen in 64% by diary review. Of those, 41% sought attention. The mean onset was 9 days (1-31), the time ill before being seen was 39 h (2.5 -141), and the number of unformed stools was 5.7 (2-17).

Toll-Like Receptors were represented in the results of the SNPs analysis. TLRs are evolutionarily conserved receptors that play a key role in the induction of immunity and inflammation. At least 11 TLRs have been identified, a picture complicated by their ability to function in complexes. The TLRs are relatively specific for bacterial, viral, and parasitic products, for example, TLR4 is a receptor for LPS, and TLR5 is specific for flagellin. TLR 5 is present in intestinal epithelial cells, dendritic cells, microvascular endothelial cells and monocytes. Basolateral enterocyte activation of TLR5 leads to phosphorylation and nuclear translocation of the transcription factor NFκB. Two TLR5 SNPs were studied, 1174 C →T and 1775 A →G. SNP 1174, which was significantly associated with increased diarrhea (p=0.04), results in $TLR5^{392STOP}$ with a loss of function and has previously been associated with hypo-responsiveness and mortality due to *Legionella.*

The study of fecal chemokines showed that of IL-1 β, IL1r α, TNFa, IL-6, IL-8, and INF γ , IL-8 was the most highly induced. Of *Shigella,* EAEC, ETEC, and *Salmonella, Shigella* was the most inductive. Interleukin-8 is a chemokine that attracts neutrophils to sites of inflammation and activates macrophages; elevated fecal levels occur in response to infection during bacterial diarrhea and from enterocyte cell lines exposed to these pathogens. Significantly increased IL-8 production has been described in response to AggR alone and combinations of virulence determinants. A polymorphic allele (-251A) of IL-8 has been implicated to a trend toward increased response of PMN to LPS. In addition, the -251A allele increases susceptibility and severity of RSV bronchiolitis, increases susceptibility to TB, and decreases susceptibility to colon cancer. The IL-8 -251 T→A SNP is significant in travelers' diarrhea, however fecal levels of IL-8 vary greatly according to genotype from ~135 pg/ml (AA), to ~20 pg/ml (AT) and 0.5 pg/ml (TT). Intriguingly, 44% of the AA travelers, 66% of the AT, but 0% of the TT study travelers who visited clinics during their stay had EAEC identified from stool culture, suggesting a significant protective effect of the TT genotype against EAEC.

The immunoregulatory cytokine Interleukin-10 is anti-inflammatory, leading to decreases IL-1, TNF-α, IL-8, and increased levels of IL-1RA, TNFsr. In addition, IL-10 stimulates NK cells, enhances production of IL-4 and IgA. IL-10 SNPs in the promoter region have been associated with sepsis, coronary artery disease, increased inflammation in *H. pylori* infection, susceptibility to HIV infection, psoriasis, and IBD. The -592 IL–10 SNP only appeared to be significantly associated with cryptosporidiosis among the enteropathogens tested, with a significantly greater number of cases occurring among the CA genotype compared to the AA or CC homozygotes. Overall, the non-CC individuals were more likely to suffer from diarrhea but less likely to present at the clinic. The most significant protection was seen against cryptosporidiosis, with the CC genotypes protected ($p=0.001$) relative to non-CC genotypes. The SNPs in IL-8 and IL-10 appear to interact, leading overall to major host-dependent response and vulnerability to travelers' diarrhea.

RESAERCH NEEDS:

- Genetic polymorphism determination in diarrhea studies conducted among pediatric and other populations both in developed and developing countries The significance extends to both acute and chronic infections caused by enteric pathogens, including the opportunistic infections occurring in the HIV infected
- Genetic polymorphism determination in vaccine safety and efficacy studies. These data may be informative regarding reactogenicity, intussusception and vaccine failure among diverse populations in different geographical settings, and may facilitate the determination of optimal schedules
- Determine the possible role of host genetics on a range of post-diarrhea complications such as Guillian-Barre Syndrome, reactive arthritis, persistent diarrhea, malnutrition and irritable bowel syndrome